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Impacts of salinity on germination and early seedling growth of bean plants (bean *phaseolus*)

Firew Admasu Hailu* and Ahmedin Jeylan
Department of Biology, College of Natural and Computational Sciences, Dilla University, Ethiopia

Corresponding author: firew.admasu@gmail.com

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ABSTRACT

Aim: The study was carried to evaluate the effects of salinity on germination and early seedling growth of Bean plants.
Materials and Methods: The study was conducted at Dilla University, in the laboratory of Biological sciences on 21 groups out of which 20 were experimental with different salt concentrations ranging from 0.05 M to 1.00 M with 0.05 M difference and one control group. Each group contain three bean seed measuring its weight using electronic balance machine, sterilized with 70% Alcohol solution for 15 seconds, rinsed with distilled water, placed in separate Petri dish using a forceps, 50 ml of solution were added to each Petri dish with different concentration, all Petri dishes were covered with lids and kept into incubator at room temperature for 17 days, germinated seed were counted, seedlings root and shoot length were measured using a ruler. Finally, the Bean was transferred to non-saline condition, weight of germinated Bean were measured to compare with their normal weight and to determine the effects of salt on seed weight, which was conducted in triplicates. All necessary data were taken, analyzed and interpreted.
Results: While the concentration of salt increased, the Bean plants were extremely affected, germination rate decreased and the terminal weights of seed were become lower than the initial weight due to the absorption of water by seed.
Conclusion: The rate of germination decreases when the salt concentration increased and late growth of length of root and shoots when the salt dosage is highest and also the weights of seed reduced as concentration of salt is raised. Generally, as the concentration of salt is increased the Bean plant is extremely affected.

Keywords: *Early Seedling, Salinity Effect, Salt Concentration, Seed Germination*.

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Introduction

Plant productivity is affected by limited factor known as soil salinity (Szabolcs, 1994). The growth of crop plants are exposed to different environmental stresses such as salinity and drought limit and also due to these from world cultivated lands estimated more than 20% contain high salt levels to cause salt stress on crop plants (Reynolds, 1995). Seedlings has vulnerable life cycle of seed plants and their germination determines when seedling growth begins on the soil salt concentration (Leorcher, 1974). The United Nations Environment Program (UNEP) salt stressed estimates that 20% of the agricultural land and 50% of the cropland in the world (Flowers and Yeo, 1995) and also salinity imposes environmental problems that affect grassland and accessibility of animal feed in dry and semi-arid regions (El-Kharbotly *et al.,* 2003).

Salt pressure adversely affected the growth and productivity of plant during all developmental stages and salinity decreases seed germination, retards plant development and reduces crop yield (Epstein *et al.,* 1980). Maximum germination of seeds of most plant species reach in distilled water and sensitive to elevated salinity at the germination and seedling phases of development (Gulzar *et al*., 2003).

In many plant species, seed germination and early seedling growth are the most salinity sensitive stages to attain maximum germination without NaCl and salinity elevated at the germination and early establishment phases (Khan *et al.,* 2000). Many physiological aspects of plant shoot growth and dry matter are reduced by salinity (Rahman *et al.,* 2008) and it also affect seeds of germination by creating osmotic potential which prevent water uptake, or by toxic effects of ions on embryo viability (Loercher, 1974; Houle *et al.,* 2001). Osmotic potential reduction in salt stressed plant is a result of inorganic ion (Na+, Cl- and K+) and accumulations of complete organic solute (Hasegawa *et al.,* 2000). Increased salinity causes reduction in germination percentage, rate, both length and weights of root and shoots (Jamil *et al.,* 2006). Growth of shoot reduced by salinity due to inhibitory effect of salt on cell division and growing point (Munns, 2002). Soil salinity is one of the major constraints in beans production due to chloride and sulfate accumulation, some have a very high germination capacity and seedling growth on saline soil, while others have well germinate, but with a very poor seedling growth in saline soil (Asfaw and Ghosh, 2000).

In crop plants, germination and seedling characteristics are the most feasible criteria used for selecting salt tolerance (Jamil and Rha, 2004) and the germination percentage, rate, and seedling growth are important growth parameters (Khodarahmpour *et al.,* 2012). Bean Phaseolus species is salt sensitive and its yield highly reduced by effects of salinity wide ranging from germination to stages of vegetative, fruiting, and padding; and salinity tolerance of Phaseolus species differs from one genotype to another (Rupela, 1999). Germination is the first stage of a plant's life cycle for establishment of a crop, oxidization takes place within the seed to breaks down storage proteins to obtain energy and amino acids necessary for the development of plant (Almansouri *et al.,* 2001; Ahmed *et al.,* 2019). The legume family is the third largest of flowering plants (Morris *et al*., 2003; Lewis *et al.,* 2005). Economically, legumes represent the second most important crop plants after Phocaea family, about 27% of the world's production of crops (Graham and Vance, 2003).

In Africa, Ethiopia is the second well known of producer of food legumes next to Egypt (Lewis *et al*, 2005). However, about 10,608 half of the total land of Ethiopia's is affected by salinity and may reduce the yield (Geressu, 2011) in both arid and semi-arid regions. Moreover, saline in semi-arid parts of irrigated lands are increase and so rotating to new scenarios of food production for the fast growing population is important (Turan *et al.,* 2007). Beans are an important source of protein in East and Great Lake of Africa, which play an vital role in human nutrition, providing as much as 45% of the total protein consumed (Allen *et al.,* 1999). In addition, it plays an important role in soil fertility, particularly in arid regions (Saxena, 1990). Therefore, in the countries like Ethiopia where legume plants are mainly grown for human food consumption for the rapid population growing, focus should be given on the development of salt tolerant crops species and cultivate is important to reach their food security. In addition, there are a number of problems caused by salinity on plant germination, growth and productivity. In order to improve agricultural productivity, improving salt tolerance of crop plants has great potential. Agriculture is backbone of the country's economy but the sector is highly influenced by natural, social and human induced problem. In Ethiopia Bean Phaseolus species is the main cash crops, grown by small farmers and used as a major food legume in many parts of the country. This study was carried out to investigate salinity effect on both germination and early seedling of Bean plants; because of identification of salt tolerant species have huge value for agriculture.

Materials and Methods

Description of Study Area:- The study was conducted in Dilla University main campus, at the laboratory of biological sciences department, located in southern nation, nationalities and people of regional state (SNNPR), at about 360km to south from Addis Ababa, Ethiopia.

Materials used for the study: The following materials /chemicals and instruments was used for this research work, these are; Petri dish (9.5 cm diameter), Sodium chloride (NaCl), Bean seed, 70% Alcohol, Distilled water, Balance machine, Laminar air flow.

Treatment and solution preparation: Before the beginning of the experiment, the solutions were prepared from sodium chloride (NaCl) and distilled water. There are 20 experimental groups with different salt concentrations and one control group without any addition of salt. The experimental groups are labeled as 0.05M, 0.10M, 0.15M, 0.20M, 0.25M, 030M, 0.35M, 0.40M, 0.45M, 0.50M, 0.55M, 0.60M, 0.65M, 0.70M, 0.75M, 0.80M, 0.85M, 0.90M, 0.95M, 1.00M and the controlled group is 0M. The solutions were left for 6 hours until completely dissolved. The concentrations of the solutions were prepared by using molar conversion method as follows.

- *M= N/V. Where, M is molecular mass,*
  N is number of mole, and
  V is volume.
- N= molar mass/ given mass (mass of NaCl)
- Molar concentration = mass of NaCl/molecular mass x Volume.
- Mass of NaCl= Molar concentration x molecular mass x Volume.

The salt (NaCl) was added to 50ml of distilled water depending on the needed concentration. The salt concentration was prepared as follows (Table 1).

Table 1. Solution preparation

No	Concentration (M)	Amount of NaCl added (g)	Amount Distilled water (ml)
1	0.00	0	50
2	0.05	0.15	50
3	0.10	0.29	50
4	0.15	0.44	50
5	0.20	0.58	50
6	0.25	0.73	50
7	0.30	0.87	50
8	0.35	1.02	50
9	0.40	1.27	50
10	0.45	1.31	50
11	0.50	1.46	50
12	0.55	1.60	50
13	0.60	1.75	50
14	0.65	1.90	50
15	0.70	2.04	50
16	0.75	2.18	50
17	0.80	2.34	50
18	0.85	2.50	50
19	0.90	2.65	50
20	0.95	2.77	50
21	1.00	2.92	50

All the required Petri dishes was prepared as germination chambers and labeled based on salt concentrations, then disinfected with 70% alcohol and UV to make free from microorganisms.

Experimental Design: The Bean seeds and Salt (NaCl) were bought from the Dilla town (Market) to the laboratory and sieved to eliminate broken, small and defected seeds. The normal sixty three (63) seeds were taken and randomly divided in to 21 group (each group has three seed). Each seed group's weight were measured using electronic balance machine, and then the seeds were sterilized with 70% Alcohol solution for 15 seconds and rinsed with distilled water. Each of experimental seed groups were placed in a Petri dish (9.5 cm diameter) using a forceps (three in one), then, 50ml of prepared salt concentration was added to it by following the amount of labeled concentration and 50ml of distilled water is added in to the control group and finally, all of them covered with lids and kept into incubator at room temperature (24 ± 2 ^{0}C) and the experiment lasted at 17 days. Germination count was made on 17th day and the germinated seeds identified based on the appeared and visible shoot and root. Then, length of both root and shoot of seedling were measured using a ruler. Finally, the Bean was transferred from saline condition (Petri dish) to non-saline condition in order to measure the weight of germinated Bean to compare with their normal weight and to determine the effects of salt on seed weight. The experiment was conducted in triplicate to increase the validity of the result.

Data collection and Analysis: All necessary data including measuring the length of shoot and root of beans in the different salt concentration and the weights of seed before and after germination were recorded. Mean and percentage of statistical data analysis was performed to identify the differences between control growth and experimental growth and within the experimental group each other. The obtained data were analyzed and interpreted in the form of percentage, graph and tables. The parameter measured was included percent germination, length of shoot and percentage of shoot length, length of root and percentage of root length in each concentration and the weight of germinated Bean.

Results and Discussion

The result of this experimental study indicated that as the concentration of salt was increased the Bean plant was extremely affected as indicated (Fig. 1).

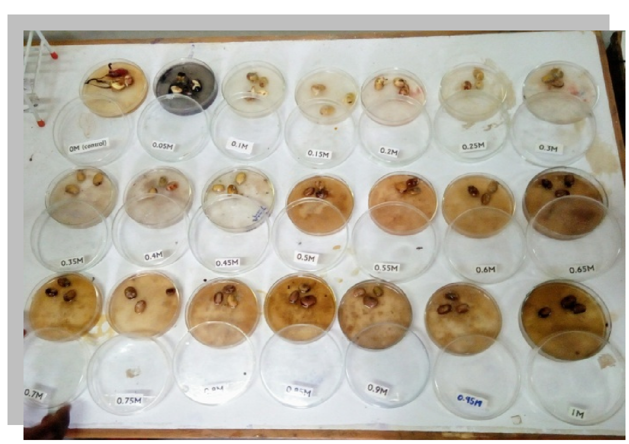

Fig. 1 Growth indication of bean plants

Based on the result of this experimental observation as indicated above in figure 1, Up to 0.20M there is certain amount of observable seed germination. However, above 0.20M there is no any observable seed germination. As the higher concentration of salt was limited in water absorption, and slower in germination rate. In the salt concentration from 0.25M - 0.35 M the seed coat broken was seen for starting germination but become unseen as salt concentration were increased and there's no observable shoot and root germination. There was significant differences observed between treatments for germination rate and which means that the germination rate decrease when the salt concentration is increased and late for the highest salt dosage of the study seeds.

Effects of salinity on the growth of root and length of shoot seed: Both root and shoot length was measured by using ruler after 17 days exposure and the maximum length of the shoot result were recorded on the control group, which is 58mm and it was gradually decreased to 39, 19 , 10 and 9mm in the salt concentrations increases from 0.05, 0.10, 0.15 and 0.20 M, respectively. Similarly, the length of the root reduced in all the treated Bean seeds when compared to the control and 35 mm was recorded in the control and gradually reduced as the concentration of the salt is increased and the recorded results were 12, 10, 6 and 0mm, respectively. The maximum and minimum results of all the study are summarized (Table 2) and (Fig. 2).

Table 2. The effect of salinity on the root and shoot length

№	Concentration (M)	Length of Root (mm)		Length of Shoot (mm)	
		Maxim	Minim	Maxim	Minim
1	0 (Control)	35	18	58	21
2	0.05	12	2	39	11
3	0.10	10	2	19	17
4	0.15	6	0	10	6
5	0.20	0	0	9	6

Fig. 2 Growth of bean plant

The percentage growth of shoot length was also reduced from 100% in the control to 67.24, 32.75, 17.24 and 15.52% in the salt concentrations of 0.05, 0.10, 0.15 and 0.20M, respectively and summarized (Fig. 3).

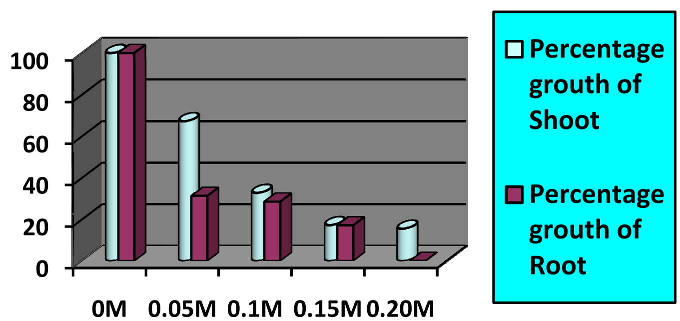

Fig. 3. The effects of different salt concentration on the growth of root length and shoot length by percentage

The effects of salinity on the weight of seed: The weight of seed was recorded by Mean (measuring three seeds together that were added in one Petri dish, then divided for three). The terminal weights of seed were become lower than the initial weight as the concentration of salt is increased. This is due to seed inhibition of water absorption from their surrounding by salt and salt absorption of moisture from seed. The salinity effects on the weight of seed of 20 experimental and a control group are summarized (Table 3).

Table 3. The effects of salinity on the weight of seed

№	Concentration (M)	Initial weight of seed (g)	Terminal weights of seed (g)	The difference (effects)
1	0 (control)	1.84	2.38	+0.54
2	0.05	1.66	2.19	+0.53
3	0.10	1.63	1.75	+0.12
4	0.15	1.67	1.64	-0.03
5	0.20	1.84	1.61	-0.23
6	0.25	1.80	1.31	-0.49
7	0.30	1.78	1.29	-0.49
8	0.35	1.87	1.20	-0.67
9	0.40	1.78	1.15	-0.63
10	0.45	1.88	1.10	-0.78
11	0.50	1.76	1.14	-0.62
12	0.55	1.80	1.17	-0.63
13	0.60	1.77	1.15	-0.62
14	0.65	1.79	1.13	-0.66
15	0.70	1.79	1.14	-0.65
16	0.75	1.78	1.16	-0.62
17	0.80	1.76	1.10	-0.66
18	0.85	1.82	1.17	-0.68
19	0.90	1.85	1.23	-0.62
20	0.95	1.88	1.20	-0.68
21	1.00	1.80	1.14	-0.66

As seen above on the table 3 about different concentration, initial and terminal weight of experimental bean seed of 21 groups with their differences indicate that, the terminal weights of seed was become decreased as salinity increased up to 0.45M. This indicated the effects of salt on the weight of seed. The seed in the salt concentration from control up to 0.45M the seed coat were broken. This indicate the internal nutrient (material) are still upon starting the germination at low germination rate due to salt effects, and some are discharged out through the broken coat of seeds and this may a reason to loss the weight of seed. In addition, from 0.50M-1.00M the terminal weights of seed are nearly on the stationary as salinity increased, but lower than the original seed weight. The color of seed was changed and become black as concentration of salt is increased, especially above 0.50M due to accumulation of salt and inhibitory effects of the solution low osmotic potential and also the Bean seed were died as salt concentration is increased.

In many seed plant species, germination and early seedling are the most sensitive to salinity pressure. The maximum germination in the absence of NaCl and are very sensitive to elevated salinity and early establishment phases (Khan *et al.,* 2000). Shoot growth is reduced by salinity (Rahman *et al.,* 2008). This result is similar with the current study, when salt concentration increased, germination of seed reduced, the growth of shoot and root is affected. The result of this study is comparable with both Munns, (2002) and Jamil *et al.,* (2006). Meaning that, increasing salinity concentration can causes decrease in germination percentage and rate, length of both root and shoots and also fresh root and shoots weights (Jamil *et al.,* 2006) due to the inhibitory effect of salt on cell division and enlargement in the growing point (Munns, 2002). The current studies showed that, the root and shoot length and also weight of Bean *Phaseolus* spp, were affected by different salt concentration levels.

The result of the current study also supported by different researchers such as Epstein *et al.,* (1980), Ghoulam and Fares, (2001), Munns, (2002), and Gulzar *et al*., (2003). Salt stress critically affected plant growth and productivity during all developmental stages by decreasing germination rate, retards the development of plant and reduces the yield of crop (Epstein *et al.,* 1980). Several investigations on most species of seed germination under salinity stress reach maximum germination in distilled water and seedling phases of development (Ghoulam and Fares, 2001; Gulzar *et al*., 2003). Increased salinity causes a significant reduction in germination percentage, germination rate, and root and shoots length as well as fresh root and shoots weights (Jamil *et al.,* 2006). Shoot growth was reduced by salinity due to the inhibitory effect of salt on cell division and enlargement in the growing point (Munns, 2002).

Conclusion

It was concluded that from control up to 0.20M there was observable seed germination, but the germination rate decreases as salt concentration increased and delayed for the highest salt dosage. The length of root and shoot as well as the weights of seed were reduced as concentration of salt is raised, due to seed inhibition of water absorption by salt and salt absorption of moisture from seed. In high salt concentration (0.5M and above) the seed were going to die and the color is changed to black because of ionic toxicity.

Generally, as the salt concentration is increased the Bean plant is extremely affected.

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